# Effective Methods for Study of Q Fever in the Americas

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INFECTIONS with *Coxiella burnetii* are asymptomatic in animals and domestic livestock throughout the world but represent a significant and widespread cause of human illness (*1*). Animal infection is already widespread, and further dissemination is facilitated through movement of livestock between countries. In some European countries, where people live in close proximity with their livestock, periodic and severe epidemics (*2*) result in thousands of human cases annually. Whether similar or appreciable illness results from the recognized animal reservoirs of infection throughout North and South America remains to be determined. Although enzootic infections do not always result in a severe disease problem (*3*), their existence is a matter of concern because the factors responsible for human cases are not yet fully understood. The necessary investigations within various unstudied areas on most continents could be quite simply performed through the application of the numerous and reliable test methods now available.

Some systematic investigations have been carried out in the United States to determine the occurrence and trends of Q fever infections among the bovine reservoirs (*4*) and to search comprehensively for associated human infections (L. Luoto and M. L. Casey, unpublished paper). From these activities basic methodologies have evolved that are adaptable to studies which must conform to customs and agricultural practices in different countries.

This report summarizes approaches and sampling methods that have facilitated area studies and presents data obtained on both animal and human infections with the simple capillary agglutination test. Findings obtained with other more sensitive test methods are reviewed along with practical considerations of their application in research.

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## Approaches to Studies

Efforts were made initially to ascertain the occurrence, geographic distribution, prevalence, and trends of Q fever infections in livestock populations. Thus, regardless of findings achieved during human studies, background data on the sources of human infections were available for future evaluations. Such baseline data on animal involvement were obtained through tests for antibody in milk or serum specimens. Existing activities and facilities within areas of study were utilized, such as agricultural and health-related programs of brucellosis or milk-quality control. Preservation of milk samples for shipment to a central testing laboratory has made it practicable to use distant sampling agencies and to mail samples from farms. Detection of antibody in milk specimens, especially that in pooled milks from entire herds, greatly facilitated investigations because collection of pooled specimens was much more acceptable to owners than the drawing of blood samples.

Sampling of human populations was simplified by relying upon serums obtainable from "pilot tubes" of blood banks and from clinics,

hospitals, and regional health departments doing diagnostic or prenatal tests. As programs developed, more direct studies of special groups under greater risk of infection were achieved through investigators making individual visits and doing sampling. If objections were encountered, especially in studies with children, Q fever skin tests were substituted and readily accepted. Initial efforts were devoted to direct, personal contacts with responsible agencies and families involved to convey assurances that findings would be confidential, to explain goals and methods, and at the same time to solicit their active interest and cooperation in the programs. Subsequent efforts to report and discuss general findings with participants were essential to their continued participation and to the effectiveness of the research.

Reliable and simple methods for testing large numbers of specimens are essential for the performance of large-scale surveys, whereas more cumbersome tests can be used for detailed examination of small numbers of specimens. Among the various tests applied during Q fever epidemiologic studies have been the complement fixation (CF) tests (*5*), microscopic agglutination (*6*), capillary agglutination (CA) (*7–9*), and the standard tube agglutination tests (*10*). More recently, research has provided the skin test (*11*, *12*), and the mouse neutralization (MN) (*13*), the agglutination resuspension (AR) (*14*), and the radioisotope precipitation (RIP) tests (B. H. Hoyer, R. K. Gerloff, L. Luoto, and D. B. Ritter, unpublished paper and reference *15*), which are applied to serum specimens. Previously recognized limitations of some tests, or those becoming apparent during recent studies, present certain difficulties in surveys. Of those methods mentioned, the capillary agglutination test which has been evaluated for detection of antibody in many types of specimens appears adaptable for a wide variety of studies.

### Capillary-Tube Agglutination Method

The recorded characteristics of the capillary-tube agglutination test have been confirmed by investigators on several continents. The procedure, performed in small tubes to conserve antigen, has been evaluated for examination of bovine, human, and other serums as well as on individual raw or pooled milks from entire dairy herds. It can be performed rapidly on several hundred specimens per hour. Readings are based on the formation of colored agglutinates, or of colored cream layers in milks, in specimens which contain antibody to *C. burnetii*. Screen tests on milk or serum can be read after 4 hours or overnight, whereas titrations are read after 24 hours; furthermore, CA test reactions remain readable for many days. Inactivation of serums should be avoided since it alters the agglutinability reaction. CA tests on serums other than human, bovine, ovine, or guinea pig should be interpreted with caution until adequately evaluated for such use.

Capillary agglutination tests of herd milks previously studied (*16*) and of individual milks were evaluated for specificity, reproducibility, and sensitivity during intensive epizootiologic studies in Montana (L. Luoto, unpublished data). They were found to follow regular patterns and to provide effective methods for study of bovine infections. These findings, to be

**Table 1. Isolation of Coxiella burnetii through inoculation of hamsters with herd milk pools previously tested by the capillary agglutination method**

| CA titer of milk injected | Number isolations [1] in hamsters/ number tested in hamsters | | | |
|---|---|---|---|---|
| | Montana herds | Wisconsin herds [2] | Total | |
| | | | Number | Percent |
| 0 | [3] 1/31 | 0/30 | 1/61 | 1. 6 |
| 1:1 | 17/28 | -------- | 17/28 | 62. 9 |
| 1:2 | -------- | 2/5 | 2/5 | -------- |
| 1:4 | 16/20 | 7/8 | 23/28 | 82. 2 |
| 1:8 | 31/35 | 8/9 | 39/44 | 88. 8 |
| 1:16 | 26/27 | 8/9 | 34/36 | 94. 4 |
| 1:32 | 15/15 | 7/8 | 22/23 | 95. 6 |
| 1:64 or more | 5/6 | 10/11 | 15/17 | 88. 3 |
| Total | 111/131 | 42/50 | 152/181 | 84. 0 |
| Percent | 84. 0 | 84. 0 | -------- | -------- |

[1] A hamster serum CF titer of 1:32 or greater 6 weeks after intraperitoneal injection of 1 ml. of herd milk sample represents a serologic isolation (numerous strains were grown in fertile eggs).

[2] Data provided by Dr. Henry Wisniewski, Milwaukee (Wisconsin) City Health Department.

[3] Two months previously this herd had a CA milk titer of 1:2.

**Table 2. Results of capillary-tube agglutination tests performed on herd milk pools within a Q fever endemic area in Idaho, November 1958 through June 1959**

| Herd [1] | November 1958 | December 1958 | January 1959 | February 1959 | March 1959 | April 1959 | May 1959 | June 1959 |
|---|---|---|---|---|---|---|---|---|
| 1 | [2] + | + | + | + | + | + | + | + |
| 2 | 0 | 0 | + | + | + | + | + | + |
| 3 | + | + | + | + | + | + | + | + |
| 4 | + | + | + | 0 | + | + | 0 | + |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | + | + | + | + | + | + |
| 8 | 0 | 0 | 0 | 0 | + | + | + | + |
| 9 | 0 | + | + | 0 | 0 | + | + | 0 |

[1] Representative of 99 herd milks submitted monthly on grade A herds for bacteriological examinations by the Idaho State Health department. (Data provided by Darrell Brock, chief, Bureau of Laboratories.)
[2] Agglutination occurred in test of undiluted milk or in diluted specimens.

fully reported in a subsequent paper, confirmed the correlation of antibody and the presence of the causative agent also detected in individual milks, since 84.0 percent of CA positive herd milks contained *C. burnetii* (table 1). Isolations were achieved in 60.5 percent of 28 herd milks which reacted in CA tests only at undiluted levels, and in 92 percent of high titered milks. The antibody levels reflected levels of infection within the herd as well as titers of individual milks, and they even enabled detection of single positive cows among 30 lactating animals.

The consistency of herd infections, as indicated by tests on herd milk pools, was also defined in earlier studies performed in Idaho where more than 50 percent of the herds contained infected animals (table 2). Large positive herds rarely reverted to a negative status; in some family-type herds, the sale or cessation of lactation of the occasional positive cow can change the status of the herd. Valid CA tests were possible for at least 7 days after mailing of milk specimens to which preservative (0.3 ml. of formalin diluted 1 to 5) had been added. Herd samples collected over a 2-week period and preserved with mercuric chloride tablets as in brucellosis test programs were frequently utilized, as were herd milk samples collected in weigh-in tanks at milk depots. Tests on goat milks and serums, while based on limited samples, appear to be effective survey tools; sheep milks were not found to be such sensitive indicators of presence of antibody as were serum specimens (L. Luoto, unpublished data). Consequently, availability of a simple test for antibody in animal specimens makes practical the large-scale search for the existence or for the development of changes in infection status among reservoirs of the disease in most agricultural areas.

The CA test has also been successfully employed in several statewide surveys for Q fever antibody in human serums. During studies in Idaho, an endemic area, 1.9 percent of 12,413 routinely collected health department and blood bank serums were found positive. Prevalence of antibody in human beings varied from 0 to 6 percent in the counties tested and correlated closely with the areas of known bovine infection among 13,849 dairy herds tested; 22.2 percent of the herds contained infected animals. Several exceptions to this correlation occurred in Idaho counties known to have a high prevalence of in-

**Table 3. Capillary-agglutination tests for Q fever performed on human serums in Montana surveys**

| Type of specimen | Number tested | Number positive [1] | Percent positive |
|---|---|---|---|
| Blood bank (78 percent male donors) | 3, 261 | 79 | 2. 4 |
| Routine diagnostic tests (70 percent female donors) | 8, 016 | 84 | [2] 1. 0 |
| Total | 11, 277 | 163 | 1. 4 |

[1] Agglomeration in undiluted serums.
[2] In tests of clot serums, 37, or 1.6 percent, of 2,370 were positive; with inactivated serums, 47, or 0.8 percent, of 5,646 were positive.

fected sheep flocks. Other tests on 11,277 similarly collected human serums from Montana, where only bovine infections occurred, showed 1.4 percent to be positive (table 3) and to occur in areas of bovine infection. The tests were performed as a part-time activity of two technicians over a 4-month period. Of particular interest were the higher proportion of positives among blood bank specimens taken predominantly from men who undergo greater occupational exposure to infected animals and the antibody-reaction depressant effects of serum inactivation.

### Comparisons of Test Sensitivities

Observations made during the various phases of Q fever studies in Montana showed that the selection of laboratory test methods could affect drastically findings from surveys of human or animal infections. Results of CF and CA tests of specimens from blood banks, from residents with infected dairy premises, and from vaccinated individuals (table 4) showed that capillary agglutination tests were appreciably more sensitive for detection of antibody during surveys of human serums. The CA, unlike the CF method, was of equal sensitivity in tests of the same serums performed in various laboratories with different antigens and, furthermore, was not complicated by phase I and II variations obtained with CF tests (table 5).

When serums from patients with acute cases of Q fever were examined, there were fewer clear-cut differences between the two test methods (L. Luoto, unpublished data). Serums obtained 2 to 3 weeks after onset of illness react sooner and to a higher CF titer than do serums in CA tests; since occasional diagnostic serums may be negative by either test, both methods have been employed to detect antibody in such specimens. Antibody in serums drawn at 3 to 4 weeks after onset, especially antibody at low levels, is readily detected by CA. The reported equal sensitivity of CA and CF tests of animal serums, except for hamster serums (*9*, *10*), remains valid except for two modifications: CA titer develops more slowly, by 30 days, in serums of experimentally inoculated guinea pigs, and some CF test antigen strains fail to fix complement in tests of sheep serums.

Comparative data accumulated during recent studies in Montana dictate the need for application of newer and more sensitive test methods to measure past human exposure to *C. burnetii.* The relative insensitivity of the widely employed CF and CA tests became apparent (table 6) when AR, MN, and RIP tests identified a 10 times greater number of sensitized persons among naturally exposed and vaccinated individuals. The relative sensitivities of the various test methods were very similar in both study groups. The findings, based on the vaccinated group, reflect the time interval between known

**Table 4. Detection of Q fever antibodies by complement fixation and capillary agglutination tests on human serums**

| Test result category | Source of specimen | | | | |
|---|---|---|---|---|---|
| | Blood bank donor | Resident of infected premises | Vaccinated | | Suspected case |
| | | | Laboratory worker [1] | Prison volunteer | |
| Tested both methods | 1,174 | 53 | 51 | 120 | 74 |
| Total positive by CA | 26 | 17 | 48 | 81 | 56 |
| Total positive [2] by CF | [3] 12 | 3 | 25 | 7 | 61 |
| Positive by both | 11 | 2 | 25 | 7 | 50 |
| Positive CA only | 15 | 15 | 23 | 74 | 6 |
| Positive CF only | 1 | 1 | 0 | 0 | 11 |

[1] The 51 laboratory persons were under subsequent exposure to *C. burnetii.*
[2] Reproducible agglomerates in undiluted serum; fixation at 1:8 or higher dilution (in laboratory of Dr. D. B. Lackman, Hamilton, Mont.).
[3] Only 5 were positive by CF in another laboratory.

**Table 5. Representative examples of Q fever antibody titers indicated by complement fixation and capillary tests on various types of serum specimens**

| Serum sample numbers | Titers of blood bank specimens by— | | | | Titers of serums of suspect cases by— | | | Titers in serums of vaccinated persons by— | |
|---|---|---|---|---|---|---|---|---|---|
| | CA test in laboratory | | CF test in laboratory | | CA test in laboratory | | CF test [1] in laboratory C [4] | CA test in laboratory B | CF test [2] in laboratory B |
| | A | B | A | B | C | B [3] | | | |
| 1 | 1 | 1 | 0 | 0 | 32 | 32–64 | 256 | 16 | 32 |
| 2 | 16 | 16 | 128 | 128 | 64 | 64–32 | 256 | 8 | 32 |
| 3 | 64 | 32 | 128 | 32 | 64 | 128–128 | 128 | 32 | 256 |
| 4 | 1 | 1 | 0 | 0 | 1 | 1–1 | 128 | 1 | 0 |
| 5 | 0 | 0 | 4 | 0 | 32 | 32–32 | 256 | 8 | 0 |
| 6 | 4 | 4 | 16 | 0 | 16 | 16–32 | 64 | 16 | 0 |
| 7 | 1 | 0 | 16 | 0 | 0 | 0–0 | 4 | 64 | 0 |
| 8 | 16 | 8 | 32 | 0 | 16 | 32–32 | 32 | 32 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0–0 | 0 | 0 | 0 |

[1] Showed antibody to phase II antigen.
[2] Vaccinated exposed laboratory workers reacted to phase I or phase II antigen, depending on time since antigenis stimulation.
[3] Titers in tests with California and Ohio strains of CA antigens.
[4] Laboratory B results in CF tests invalid because nonspecific results (titers 1:1,024 or greater) while controls appeared valid.

exposure and application of the various tests. The decay of serum antibody observed during both the vaccination studies and the infected premises study explains some of the lower CF positive rates. Detection of agglutinating antibody by the resuspension and radioisotope methods, which also measure antibody detectable by CA tests, is apparently more sensitive due to larger quantities of reagents in one and the use of vastly more sensitive methods for detection of agglutinates in the other. Positive skin tests on vaccinated persons developed rather late, as a result of delayed hypersensitivity reaction that persisted for long periods. The time of initial appearance and the persistence of positive skin tests among naturally exposed persons was not studied; laboratory-contracted cases result in positive skin tests only after several months.

**Table 6. Detection of human sensitization to Coxiella burnetii by various test methods**

| Tests applied | 53 persons living on infected premises [1] | | 33 vaccinated persons [2] | |
|---|---|---|---|---|
| | Number positive | Percent positive | Number positive | Percent positive |
| Complement fixation | 3 | 5.7 | 2 | 6.3 |
| Capillary agglutination | 17 | 32.6 | 11 | [3] 33.3 |
| Skin test | 18 | 34.0 | 32 | 97.0 |
| Agglutination resuspension | 26 | 49.1 | 20 | [3] 62.5 |
| Mouse neutralization | 35 | 66.0 | 29 | 87.9 |
| Radioisotope precipitation | 41 | 77.4 | 28 | 84.9 |

[1] Tested at undetermined interval after farm exposure.
[2] Tested 10 months after subcutaneous vaccination.
[3] At 1 to 2 months postvaccination, 63 percent of vaccinees were CA positive and 85 percent were positive by AR test.

## Practical Considerations of Test Methods

Review of the attributes or limitations of the various tests available aids in their selection during the design of studies, since many possess characteristics that limit their usefulness. Only the CA tests have been evaluated for use in survey of herd or individual milks during studies of animal infections; they are ideally suited for large-scale testing of human serum specimens as well. Attributes of the method, which can be rapidly performed by almost any-

one even outside of laboratories, have been referred to in previous paragraphs. The problems associated with performance of CF tests for Q fever (*17*), reproducibility of test results and standardization within and between laboratories, are complicated by differences in the reactivity of antigens prepared from various strains. Such problems are compounded by the complex technique, the need for highly trained personnel, even in small programs, and the reliance upon phase I and II reagents to detect such variations in antibodies produced (*18*). The microscopic agglutination test, with sensitivity approximating CA tests (*10*), involves a considerable number of manipulations in the inactivation, drying, fixation staining, and microscopic readings on each specimen but appears best suited for detailed examination of small numbers of specimens. The standard test-tube and agglutination resuspension tests require such large amounts of antigen that their use is precluded in extensive testing programs. Also the agglutination resuspension test, although somewhat more sensitive than the CA test, must be performed on fresh or centrifuged serums to insure specificity of reactions.

Three recently developed test methods present appreciably greater sensitivity along with other attributes that warrant consideration during the planning of human surveillance studies. Skin tests performed on human beings by intradermal injection of dilute suspensions of inactivated *C. burnetii*, with reading for measurable induration a week later, are effective indicators of previous exposure to and sensitization by the agent. This procedure, which is often accepted when there is objection to drawing of serum samples, necessitates an additional visit and requires medically trained personnel. The test for neutralizing antibody in serums often detects an immune response among persons negative by other tests. This procedure is somewhat cumbersome, requiring the injection of mice and the harvesting and reading of spleen smears, but is ideal for critical studies of infections among small groups of individuals, since neutralizing antibody generally appears early and persists. Another new test, the radioisotope precipitation test, a Coombs type of agglutination test using antihuman gamma globulin, as applied in poliovirus studies (*19*), appears to be most promising and the most sensitive of all tests applied to population studies (*15*). Titer readings range to very high levels only among infected or sensitized persons, and provide readily interpretable laboratory evidence. Although it requires use of radiolabeled antigen, gamma globulin, and counting equipment, these are easily prepared and applied and the method is readily adaptable to large-scale studies. This test procedure is reliable and simple, once the procedures have been established, and can convert the art of Q fever serology into a highly scientific procedure.

From this résumé of available data it becomes apparent that the design of Q fever studies in various areas will be dependent upon and somewhat influenced by the test methods available. Animal infection surveys, whether based on milk or serum sampling, can readily be accomplished with the simple and inexpensive CA test method. Such surveys will detect the occurrence, prevalence, and distribution or trends of infection among animal reservoirs, as well as locating high risk areas of human infection. Examinations for evidence of human exposure can be achieved with the CA or CF tests, the latter being especially suited for detection of acute cases of the disease. More sensitive methods such as neutralization, radioisotope, and skin tests are more difficult to perform, but they constitute better indicators of previous experience with the causative agent. The radioisotope method is uniquely sensitive and warrants serious consideration in any contemplated studies.

#### REFERENCES


(*1*) Zdrodovskii, P. F., and Golinevitch, H. M.: Q fever. *In* The rickettsial diseases. Pergamon Press, New York, 1960, pp. 372–423.

(*2*) Babudieri, B.: Q fever, a zoonosis. *In* Advances in veterinary science, Edited by C. A. Brandly and E. L. Lungherr. Academic Press, London, 1959, vol. 5, pp. 81–154.

(*3*) Luoto, L.: The epidemiology of Q fever in the United States. Amer J Hyg 74: 43–49 (1961).

(*4*) Luoto, L.: Report on the nationwide occurrence of Q fever infections in cattle. Public Health Rep 75: 135–140 (1960).

(*5*) Osler, A. G., Strauss, J. H., and Meyer, M. M.: Diagnostic complement fixation: I.A. method. Amer J Syph 36: 145–153 (1952).

(*6*) Babudieri, B.: Studies on the microscopic slide-

agglutination test for Q fever. Bull WHO 19: 981–994 (1958).
(7) Luoto, L., and Mason, D. M.: A capillary agglutination test for bovine Q fever. J Immun 71: 226–231 (1953).
(8) Luoto, L., and Mason, D. M.: An agglutination test for Q fever performed on milk samples. J Immun 74: 222–227 (1955).
(9) Luoto, L.: A capillary-tube test for antibody logical techniques for the detection and measurement of antibody to *Coxiella burnetii* in
(10) Welsh, H. H., Jensen, F. W., and Lennette, E. L.: Q fever studies. XX. Comparison of four serological techniques for the detection and measurement of antibody to *Coxiella burnetii* in naturally exposed sheep. Amer J Hyg 70: 1–13 (1959).
(11) Lackman, D. B., et al.: An intradermal sensitivity test for Q fever in man. Arch Inst Pasteur Tunis 36: 557–569 (1959).
(12) Luoto, L., Bell, J. F., Casey, M. L., and Lackman, D. B.: Q fever vaccination of human volunteers. S. The serological and skin test response following subcutaneous injections. Amer J Hyg 78: 1–15 (1963).
(13) Abinanti, F. R., and Marmion, B. P.: Protective or neutralizing antibody in Q fever. Amer J Hyg 66: 173-195 (1957).
(14) Ormsbee, R. A.: An agglutination-resuspension test for Q fever antibodies. J Immun 92: 159–166 (1964).
(15) Lackman, D. B., Gilda, G., and Phillip, R. N.: Application of the radioisotope precipitation test to the study of Q fever in man. Health Laboratory Science (APHA) 1: 21–28 (1964).
(16) Tjalma, T. J., and Braun, J. A.: Application of the Luoto capillary agglutination milk test for study of bovine Q fever. Amer J Public Health 49: 1023–1031 (1959).
(17) Stoker, M. G. P., Page, Z., and Marmion, B. P.: Problems in the diagnosis of Q fever by complement fixation tests. Bull WHO 13: 807–827 (1955).
(18) Stoker, M. G. P., and Fiset, P.: Phase variation of the Nine Mile and other strains of *Rickettsia burnetii*. Canad Microbiol 2: 310–321 (1956).
(19) Gerloff, R. K., Hoyer, B. H., and McLaren, L. C.: Precipitation of radiolabeled poliovirus with specific antibody and antiglobulin. J Immun 89: 559–570 (1962).

## Roadway Elements and Highway Safety

The relationship of traffic accidents to roadway design and traffic control has long occupied highway and traffic engineers. "Traffic Control and Roadway Elements," a 1963 publication based on a study by David W. Schoppert of the Automotive Safety Foundation, provides a comprehensive collection of research data on the subject. Based on exhaustive review and analysis of engineering studies in the United States and abroad, the study relates accident rates to such factors as traffic volume, proportion of heavy vehicles, type and width of highway and shoulder, road alinement, highway dividers, guardrails, roadside trees, ramps, protective devices at intersections and rail crossings, vehicle speeds, one-way streets, streetside parking, and provision of sidewalks.

According to the publication, past studies have explored only the relationship between the roadway factors and traffic accidents; a general theory of accident occurrence must be stated in terms of the impact of those same factors on drivers or on the traffic stream, with impact translated into likelihood of accident occurrence. The U.S. Bureau of Roads and the Automotive Safety Foundation, which jointly financed the study, hope its publication will "foster wider and more uniform application of design features of proven safety value and spur future searches for additional facts."